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The world of a worm: a framework for *Caenorhabditis* evolution

Workshop on the Study of Evolutionary Biology with *Caenorhabditis elegans* and Closely Related Species

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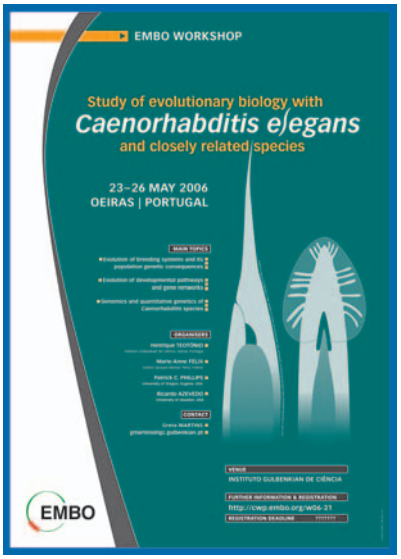

The European Molecular Biology Organization workshop on the Study of Evolutionary Biology with *Caenorhabditis elegans* and Closely Related Species was held at the Instituto Gulbenkian de Ciência, Oeiras, Portugal, from 23 to 26 May 2006. The meeting was organized by H. Teotónio, M.-A. Félix, R. Azevedo and P. Phillips.

Keywords: *Caenorhabditis elegans*; evolution; evolution of development; experimental evolution; population genetics

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Introduction

Many aspects of *Caenorhabditis elegans* biology are known in exquisite detail: all somatic cell divisions that occur from zygote to adult have been described, the synaptic connections made by all neurons have been reconstructed, the entire genome has been sequenced and the function of most predicted genes has been tested by forward-genetic screens, targeted gene-specific deletions, RNA interference or a combination of these techniques. However, less is known about the evolutionary processes that shaped the genome and the biology of this worm (Fitch, 2005). To close this knowledge gap, an exciting workshop sponsored by the European Molecular Biology Organization and the Gulbenkian Foundation brought together a diverse group of about 50 investigators, whose common interest was the evolution of *C. elegans* and its closely related species. According to the organizers, this meeting aimed to establish a network of researchers to discuss guidelines, common resources and goals for the near future. This report highlights some of the questions and recent discoveries discussed at the workshop.

Mutation rates and sex

Some life-history traits of *C. elegans* are atypical, even for a nematode. For instance, it is one of the few nematode species that is able both to self-fertilize and to outcross with males. Self-fertilization produces hermaphrodites and rare spontaneous males, whereas cross-fertilization produces equal proportions of both sexes. Although this ability comes at the price of inbreeding, one possible adaptive advantage is reproductive assurance: hermaphrodites can produce offspring in a new habitat independent of a mating partner. A second asset is the rate of reproduction: all hermaphrodite individuals are able to generate offspring, whereas only one-half of all gonochoristic individuals (that is, females) can produce offspring. What is the evidence for hermaphroditic species rapidly colonizing new habitats? A. Barrière (Paris, France) found populations of *C. elegans* in ephemeral habitats, such as decomposing fruit and dead invertebrates. These are unstable habitats that might cause high rates of population extinction and recolonization. In fact, the low level of polymorphism between strains of *C. elegans* found by Barrière, A. Cutter (Edinburgh, UK) and J. Hey (Piscataway, NJ, USA) might be consistent with a pattern of high population fluctuation (Barrière & Felix, 2005; Sivasundar & Hey, 2005), although alternative explanations (such as low outcrossing rates, recent origins of self-fertilization and recent population bottlenecks) cannot be ruled out.

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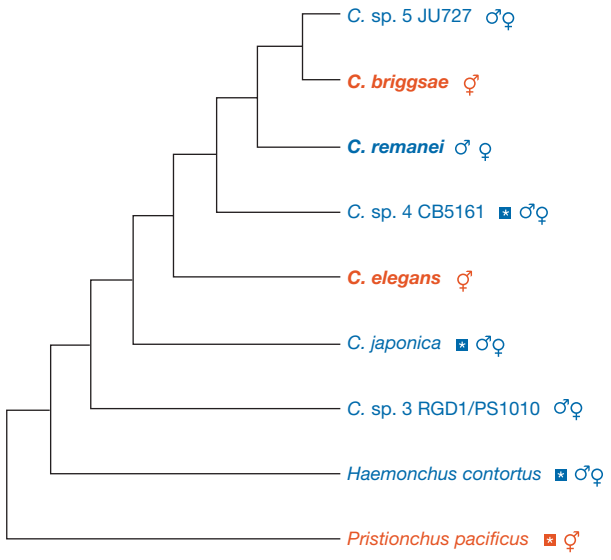

Fig 1 | Partial unscaled phylogeny of the *Caenorhabditis* genus showing species for which strains are available in culture and, thus, for experimental studies. Hermaphroditic species are shown in red and male–female species are shown in blue. Species in the queue for sequencing are indicated by a boxed asterisk; those that have already been sequenced are indicated in bold (the *Caenorhabditis remanei* genome is under assembly). *Pristionchus pacificus* and *Haemonchus contortus* are used as outgroups (Kiontke & Fitch, 2005; Kiontke & Sudhaus, 2006).

Barrière and Cutter estimate that, from levels of linkage disequilibrium, roughly one outcross occurs every 10,000 generations. However, on the basis of heterozygote frequency, the outcrossing rate is predicted to be higher (Barrière & Félix, 2005; Sivasundar &Hey, 2005). One possible explanation for the discrepancy between these calculations could be selection against heterozygotes. Indeed, E. Dolgin (Edinburgh, UK) reported that *C. elegans* natural isolates show no inbreeding depression, but do show some outbreeding depression: inbred lines generate more offspring than outbred lines. It is therefore possible that restricted recombination in hermaphroditic worms has led to co-adapted gene interactions. The disruption of these interactions by recombination results in outbreeding depression. Further support for a limited amount of outcrossing and recombination in hermaphroditic species was given by Cutter, whose study on the population structure of *Caenorhabditis briggsae* revealed that the worldwide molecular diversity of this species is also low and linkage disequilibrium is high. These characteristics limit the use of hermaphroditic *Caenorhabditis* species for mapping traits on the basis of linkage disequilibrium, as is being carried out for humans in the International HapMap Project. Taking into account the small number of polymorphic sites, and assuming that *C. briggsae* worms experience a similar mutation rate to *C. elegans*, it can be inferred that the effective population size of breeding worms is small in these hermaphroditic species. As a consequence, selection is predicted to be effective only for strongly selected traits.

H. Teotónio (Oeiras, Portugal) found evidence of quantitative genetic variation for several traits that are related to outcrossing and male performance in natural isolates of *C. elegans* (Teotonio *et al*, 2006). Of particular interest is the possible relationship between mutation and outcrossing, as an increased mutational load could favour an increase in outcrossing in the short term. Results have revealed that under high mutational loads, male frequency increases in laboratory evolution experiments independent of genetic background (see also Cutter, 2005). In a study of adaptation to laboratory conditions during 30 generations of experimental evolution, Teotónio found that in hybrid lines derived from different natural isolates, males are stably maintained at around 10%. Thus, overall, the presence of males and outcrossing is advantageous, although the specific selection pressures are not fully understood.

Although *C. elegans* males produce thousands of spermatozoa that can outcompete those of hermaphrodite worms (Ward & Carrel, 1979), very few males are found in the wild and under standard laboratory conditions. Therefore, traits associated with *C. elegans* males are expected to be under a weaker selective constraint relative to males of the dioecious species *Caenorhabditis remanei*. Experiments performed in the laboratory of K. Chow (Hong Kong, China) support this hypothesis. Mating by *C. remanei* males is extremely vigorous. At the same time, males of *C. elegans* find *C. remanei* females much more attractive than hermaphrodites of their own species (Chasnov & Chow, 2002). Chow demonstrated that the *C. remanei* female somatic gonad seems to produce (as yet unidentified) pheromones that are perceived by several neurons in the male. Interestingly, the pheromone production ceases after mating with *C. remanei* males.

Some of the molecular and developmental mechanisms underlying the evolution of hermaphroditism were addressed in the talks of D. Pilgrim (Edmonton, AB, Canada), R. Ellis (Stratford, NJ, USA) and E. Haag (College Park, MD, USA). Intriguingly, hermaphroditism has evolved from gonochorism several times within the rhabditid and diplogastrid families of nematodes (Kiontke *et al*, 2004; Herrmann *et al*, 2006). The rhabditids *C. elegans* and *C. briggsae* are self-fertile hermaphrodites, whereas the morphologically similar *C. remanei* and *Caenorhabditis* sp. 4 (a yet undescribed species) are gonochoristic species (Fig 1). One of the key innovations for the evolution of a female into a hermaphrodite occurs during gametogenesis: the gonads of hermaphroditic worms produce spermatozoa and then switch to produce oocytes (Ellis & Schedl, 2006). Many genes involved in the switch from spermatogenesis to oogenesis in *C. elegans* have been identified. For example, the F-box gene *fog-2* is essential for the initiation of spermatogenesis in hermaphrodites, but not in males. Curiously, the *C. briggsae* genome does not contain a *fog-2* orthologue (Nayak *et al*, 2005). This observation suggests that the independent evolution of hermaphroditism in *C. briggsae* involved different genes to those in *C. elegans*. Indeed, Ellis and colleagues found that the *C. briggsae germ line feminization-1* (*glf-1*) gene potentially has the same function as *fog-2* in *C. elegans*. The idea of convergent evolution of hermaphroditism in *C. elegans* and *C. briggsae* using distinct genes was reinforced in the talks of Haag and Pilgrim: the genes *feminization-2* (*fem-2*) and *fem-3*, which are required for spermatogenesis in the gonads of both males and hermaphrodites in *C. elegans*, are dispensable for spermatozoa production in *C. briggsae* hermaphrodites (Hill *et al*, 2006).

Evolution of development

One of the few phenotypes that can distinguish between *C. elegans* and *C. briggsae* is the length of the excretory duct. Compared with *C. briggsae*, *C. elegans* has a short duct and is tolerant to high salt concentrations. Previous studies have shown that *cell lineage abnormal-48* (*lin-48*) is associated with both salt tolerance and duct length (Wang & Chamberlin, 2004). *C. briggsae* does not express *lin-48* in the excretory duct cell because its promoter lacks binding sites for several transcription factors, including *cell death specification-2* (*CES-2*), *activated transcription factor-2* (*ATF-2*) and *C. elegans homeobox-43* (*CEH-43*). H. Chamberlin (Columbus, OH, USA) presented the results of mutagenesis screens in which *C. briggsae* mutants became salt tolerant and had a short duct. Interestingly, in both *C. elegans* and *C. briggsae*, salt tolerance could be uncoupled from duct length.

Natural selection acts on variation among phenotypes. Many studies indicate that phenotypes are buffered from perturbation by genotypic and environmental variation; this property is known as robustness. The *C. elegans* vulva is a well-understood stereotypical developmental system and is thus a good model in which to study robustness. M.-A. Félix (Paris, France) described developmental errors that can occur under ecologically relevant conditions and in natural isolates; for example, starvation can cause a high rate of mis-centering of the vulva in the standard laboratory strain N2, whereas it inhibits the division of some cells in the JU258 strain, a wild isolate from Madeira. In another study from the same group, C. Braendle (Paris, France) showed that mutations affecting vulva development were introgressed into different natural isolates of *C. elegans*. The mutant strains showed strong differences in phenotype, uncovering hidden genetic variation in the vulva developmental pathway. These experiments show that robustness can compensate for this variation in wild-type worms.

Evolution in the laboratory

Evolutionary trajectories in the wild are influenced by several factors, which are difficult to untangle. Experimental evolution, which refers to the study of evolution under defined and reproducible conditions, aims to control a single factor at a time. In principle, *C. elegans* makes an ideal system for this kind of study: it has a short life cycle, numerous genetic resources and tools are available, and its culture requirements are known. However, surprisingly few studies have used this worm in experimental evolution research (although see above for the work of Teotónio).

One of the few studies in this area has been performed by H. Schulenburg and colleagues (Tübingen, Germany), who have investigated experimental evolution in host–pathogen interactions using *C. elegans* and the pathogenic soil bacterium *Bacillus thuringiensis*. They observed an effect of selection on pathogen virulence, host resistance and life-history trade-offs. Furthermore, Schulenburg pointed out the role of insulin-like signalling in mediating pathogen-avoidance behaviour. This pathway has been implicated previously in physiological traits, such as stress resistance, sugar metabolism, and ageing in humans and nematodes. These new results suggest a potential link between physiological and behavioural traits mediated by the insulin pathway.

Other experimental evolution studies have used *C. elegans* to understand the dynamics, patterns and consequences of mutations. Some recent reports suggest that beneficial mutations are much more frequent than initially estimated or predicted by theory. Furthermore, compensatory epistatic mutations might be responsible for rapid fitness recovery after mutation-accumulation treatments in different organisms, such as viruses and nematodes (Burch & Chao, 1999; Estes & Lynch, 2003). Nevertheless, much remains to be addressed about the dynamics of mutational processes in *C. elegans*. D. Denver (Corvallis, OR, USA) discussed some of the questions related to mutational variation and its consequences at the fitness level; in one of his most recent projects, he studied several *C. elegans* populations that were mutant for different excision DNA-repair pathways (*MutS homologue-2* (*msh-2*), *msh-6*, *Xeroderma pigmentosum comp grp A-1* (*xpa-1*) and *endonuclease three homologue* (*nth-1*)). These mutants were subjected to 18 generations of mutation-accumulation treatment, after which more than 20 kilobase pairs of the nuclear genome were sequenced. He found evidence for mutational heterogeneity across the genome for the different repair pathways. These results have profound consequences in terms of interpreting results from molecular evolutionary analyses that rely on one or a few loci (Denver *et al*, 2006).

Unravelling the genetics of quantitative traits

M. Rockman (Princeton, NJ, USA) used a clever crossing scheme to establish highly recombinant inbred lines (HRILs) between two natural isolates: N2 and the divergent Hawaiian isolate CB4856. Single-nucleotide polymorphisms (SNPs) were genotyped at 1,451 loci for 239 HRILs and 127 wild strains, identifying 41 distinct haplotypes. The genotypes of the wild strains indicate that CB4856 has been isolated for some time; indeed, although N2 shares all of its SNPs with other wild strains, 460 SNPs are unique to CB4856.

In another study, S. Harvey (Bristol, UK) investigated the genetic basis of natural variation in dauer larva formation among strains. Worms that are in dauer are arrested in their development and are adapted for long-term survival in harsh environmental conditions. Dauer formation can be induced by several environmental stimuli, including a pheromone, low food availability and/or high temperature. Harvey showed that *C. elegans* N2 and DR1350 wild strains differed in the proportion of dauer larvae induced by different food concentrations, thereby indicating genetic variation for plasticity in dauer formation. These differences were later shown to be caused by a small number of quantitative trait loci and were also correlated with population growth rate and chemotaxis. Overall, the variation in plasticity of dauer formation seems to be part of a larger co-ordinated environmental response to food availability (Viney *et al*, 2003).

Resources: gene bits and bytes

Rockman also revealed that several sets of *C. elegans* RILs have been generated using N2 and other strains, as well as 239 HRILs between N2 and CB4856, for which 1,500 SNPs have been characterized. These resources will allow finer-scale genetic analyses that should facilitate the characterization of quantitative traits. In addition, a *C. briggsae* National Institutes of Health Genetic Map Consortium has been created and is working on the construction of a high-density genetic map, SNP software and databases (http://snp.wustl.edu/snp-research/c-briggsae) that will be useful for forward-genetics approaches.

Several groups are working to develop software and statistics that can analyse the massive amount of data generated recently by high-throughput genome projects. Among the most important tools are

gene-prediction programmes that can rapidly screen large regions of genomic DNA, and identify exon and introns. A. Coghlan (Cambridge, UK) developed Gene-o-mix, which is a programme that combines results from different gene finders based on the exons that are most conserved between species. This programme allows exon prediction with 90% sensitivity for a gene set. In addition, E. Schwarz (Pasadena, CA, USA) presented Cistematic, which is software that uses comparative analysis for the identification of potential regulatory elements in non-coding genomic sequences.

Concluding remarks

It is clear that some basic questions about *Caenorhabditis* biology remain unanswered, and additional resources need to be created or further developed. Examples include the determination of the exact ecological contexts in which *Caenorhabditis* species live to define the parameters for fitness assays; the collection of more *Caenorhabditis* species (especially from tropical regions) for speciation studies; and the establishment of molecular techniques (such as transgenics) and resources (such as species-specific microarrays) for species other than *C. elegans* to perform functional comparative genomics. Nevertheless, the prospect of having the genome sequence of about ten nematodes, which include five species outside the *Caenorhabditis* genus (*Pristionchus pacificus*, *Brugia malayi*, *Haemonchus contortus*, *Heterorhabditis bacteriophora* and *Trichinella spiralis*), as well as the inclusion of nematodes with different ecology for which genomics and genetics resources are available (Hong & Sommer, 2006), will broaden the perspective for the study of *Caenorhabditis* and nematode evolution, and promise exciting times ahead for research on *Caenorhabditis*.

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Sara Carvalho (top left), Antoine Barrière (top right) & André Pires-daSilva